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## **Telomere Biology and Cardiovascular Disease**

José J. Fuster and Vicente Andrés

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This Review is part of a thematic series on **Biological Role of Senescence in Cardiovascular Disease**, which includes the following articles:

Telomere Biology and Cardiovascular Disease

Cellular Senescence Contribution to Atherosclerosis

Mechanisms of Cardiovascular Disease in Accelerated Aging Syndromes

Mechanisms Underlying Caloric Restriction, Lipid Metabolism, and Life Span Regulation

Progenitor Cell Senescence

*Issei Komuro, Guest Editor*

## Telomere Biology and Cardiovascular Disease

José J. Fuster, Vicente Andrés

**Abstract**—Accumulation of cellular damage with advancing age leads to atherothrombosis and associated cardiovascular disease. Ageing is also characterized by shortening of the DNA component of telomeres, the specialized genetic segments located at the end of eukaryotic chromosomes that protect them from end-to-end fusions. By inducing genomic instability, replicative senescence and apoptosis, shortening of the telomeric DNA is thought to contribute to organismal ageing. In this Review, we discuss experimental and human studies that have linked telomeres and associated proteins to several factors which influence cardiovascular risk (eg, estrogens, oxidative stress, hypertension, diabetes, and psychological stress), as well as to neovascularization and the pathogenesis of atherosclerosis and heart disease. Two chief questions that remain unanswered are whether telomere shortening is cause or consequence of cardiovascular disease, and whether therapies targeting the telomere may find application in treating these disorders (eg, cell “telomerization” to engineer blood vessels of clinical value for bypass surgery, and to facilitate cell-based myocardial regeneration strategies). Given that most research to date has focused on the role of telomerase, it is also of up most importance to investigate whether alterations in additional telomere-associated proteins may contribute to the pathogenesis of cardiovascular disease. (*Circ Res.* 2006;99:1167-1180.)

**Key Words:** telomeres ■ telomerase ■ atherosclerosis ■ heart disease ■ oxidative stress ■ hypertension ■ diabetes ■ estrogens

Telomeres are special chromatin structures located at the ends of eukaryotic chromosomes that prevent the recognition of chromosomal ends as double-stranded DNA breaks, thereby protecting these regions from recombination and degradation and avoiding a DNA damage cellular response. The telomeric DNA is composed of noncoding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in vertebrates) that are extended several thousand base pairs (10 to 15 kb in humans and 25 to 40 kb in mice) and end in a 150 to 200 nucleotide 3' single-stranded overhang (G-strand overhang) (Figure 1).<sup>1,2</sup> Several specific

proteins are associated to telomeric DNA, including telomerase and the telomeric repeat binding factors 1 and 2 (TRF1, TRF2) which directly bind to the TTAGGG repeat and interact with other factors forming large protein complexes that regulate telomere length and structure. Mammalian telomerase consists of a RNA component (telomerase RNA component [TERC]) that serves as a template for the synthesis of new telomeric TTAGGG repeats by the telomerase reverse transcriptase component (TERT). Telomere components and structure has been comprehensively discussed elsewhere.<sup>2</sup>

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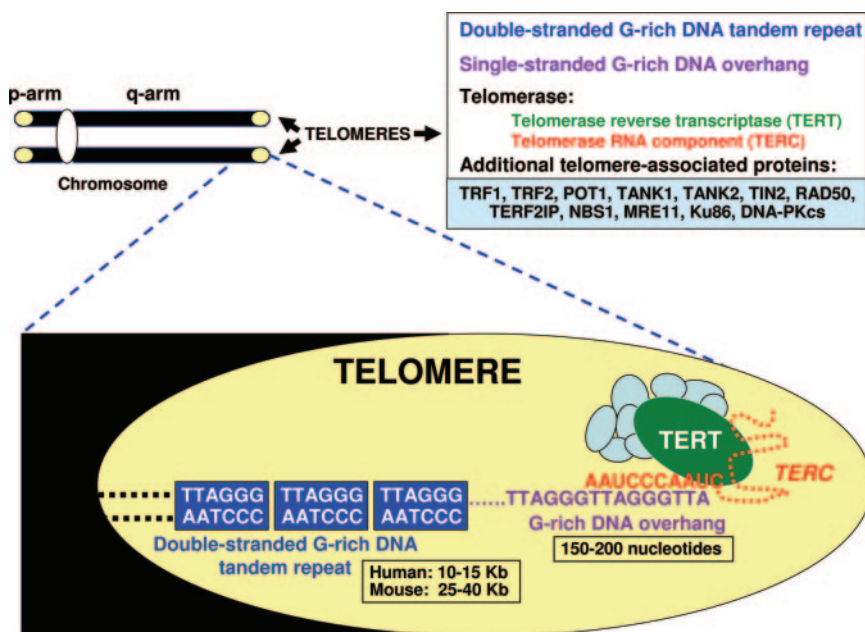
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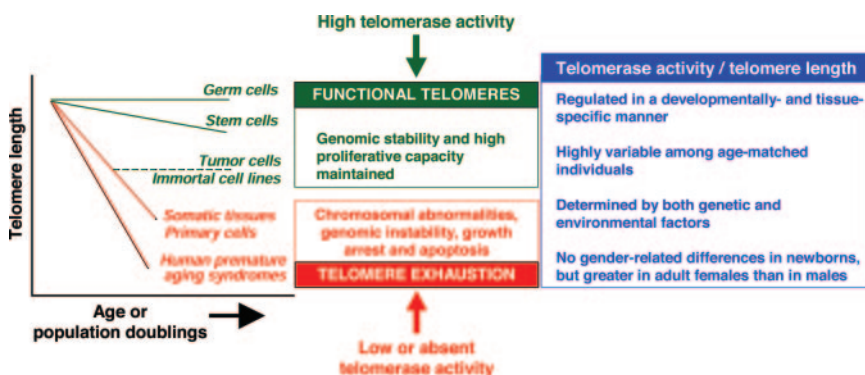


**Figure 1.** Telomere organization. Telomeres are specialized nucleoprotein structures located at the end of eukaryotic chromosomes that protect them from end-to-end fusions, thus preserving genome integrity and stability. The synthesis of new telomeric DNA repeats in dividing cells requires the activity of telomerase and additional telomere-associated proteins. Telomerase has a catalytic subunit (telomerase reverse transcriptase [TERT]) and an RNA component (telomerase RNA component [TERC]) that serve as a template for de novo synthesis of telomeric DNA. TRF1/2 indicates telomeric repeat binding factor 1/2; POT1, protection of telomeres 1; TANK1/2, tankyrase 1/2; TIN2, TRF1-interacting nuclear factor 2; TERF2IP, TRF2-interacting telomeric RAP1 protein; NBS1, Nijmegen breakage syndrome 1 protein (Nibrin); MRE11, meiotic recombination 11; Ku86, Ku autoantigen, 86 kDa; DNA-PKcs, DNA-activated protein kinase catalytic subunit.

Although telomerase expression is constitutive in most unicellular organisms, mammalian adult somatic cells typically exhibit low or absent telomerase activity and thus display progressive telomere attrition with each mitotic cycle (the so-called “end replication problem” caused by the incomplete replication of linear chromosomes by conventional DNA polymerases) (Figure 2). Accordingly, telomere length in somatic cells reflects their replicative history and can predict their remaining proliferative potential. Cells with critically short telomeres undergo chromosomal end-to-end fusions, replicative senescence, and apoptosis. In contrast to somatic cells, germ, stem, and tumor cells as well as immortalized cell lines maintain high levels of telomerase activity and thus display longer telomeres and maintain a relatively high proliferative potential (Figure 2).<sup>3–6</sup> Remarkably, TERT gene transfer reduces replicative senescence and extends the lifespan of numerous cell types, including cardiac myocytes, vascular smooth muscle cells (VSMCs), and endothelial cells (ECs) (Table 1). Telomerase-dependent transcriptional regulation of genes involved in cell growth has been recently suggested as an additional mechanism by which telomerase promotes cell proliferation independently of telomere length maintenance.<sup>7</sup>

Telomerase expression and activity and telomere length are developmentally regulated, with generally greater telomerase activity during embryonic development and low or undetectable levels soon after birth.<sup>5,8,9</sup> Human telomerase activity (hTERT) remains detectable in adult cell populations with high proliferative potential, such as activated lymphocytes and certain types of stem cells.<sup>10–12</sup> Moreover, there is also an important body of evidence demonstrating the occurrence of tissue-specific regulation of telomerase activity, both during embryonic development and in adulthood.<sup>5,9,13–15</sup> Alternatively spliced human TERT (hTERT) transcripts have been detected in several adult normal and tumor tissues and their expression that has been shown to be regulated in a tissue-specific manner during development.<sup>16–20</sup> This often leads to the expression of hTERT isoforms lacking functional reverse transcriptase domains, including dominant negative inhibitors of telomerase activity (eg, hTERT $\alpha$ ).<sup>17,18</sup>

Telomere length is highly variable among individuals of the same age, both in rodents<sup>5,8</sup> and humans.<sup>21–25</sup> Studies in twins suggest that this interindividual variability may be accounted for by genetic factors,<sup>22,24</sup> and Nawrot et al concluded that inheritance of telomere length is linked to X



**Figure 2.** Telomerase regulation, telomere dynamics, and cellular homeostasis. Most adult somatic cells have low or absent telomerase activity; hence they experience progressive telomere ablation with each mitotic cycle during normal aging and passage in culture. Once telomeres become critically short, cells accumulate chromosomal aberrations, become senescent, and eventually undergo apoptosis. Prolonged lifespan and proliferative capacity of germ, stem, immortalized, and tumor cells correlates with high telomerase activity. Several human premature aging syndromes (eg, Werner syndrome, ataxia telangiectasia, dyskeratosis congenita) are characterized by accelerated telomere exhaustion.

**TABLE 1. Cell Culture Studies Implicating Telomere Length and Telomerase in Cardiovascular Pathobiology**

Cell Type	Main Findings	References
Vascular smooth muscle cells	Increased telomerase activity correlates with VSMC proliferation	60, 63
	Hypoxia induces telomerase activity and cell proliferation in human VSMCs	59
	Telomerase inhibition rapidly abrogates VSMC proliferation	60, 61, 63
	hTERT-forced overexpression extends the lifespan of VSMCs	58, 80
	hTERT expression rescues the senescent phenotype of human plaque VSMCs despite short telomeres	62
Endothelial cells	Oxidative stress inhibits telomerase activity and accelerates telomere shortening in VSMCs	62
	Telomere attrition correlates with limited proliferative capacity of passaged human ECs	50, 149
	Telomerase ectopic overexpression augments the lifespan of human ECs	52, 80, 150
	Constitutive hTERT expression enhances the regenerative capacity of endothelial progenitor cells	89
	Fibroblast growth factor-2, but not vascular endothelial growth factor, upregulates telomerase activity and delays the onset of senescence in HUVECs	151, 152
	Estrogen induces telomerase activity by activating the phosphatidylinositol 3-kinase/Akt pathway and stimulating nitric oxide production in ECs and other cell types	118–122
	Oxidized LDLs diminish Akt and telomerase activity in ECs	126
	Chronic mild oxidative stress downregulates telomerase and accelerates telomere erosion and the onset of senescence in human ECs	127
	Statins upregulate TRF2 by a post-transcriptional mechanism and enhance the migratory capacity of endothelial progenitor cells via a TRF2-dependent mechanism	153
	Homocysteine increases the rate of telomere shortening in ECs and this effect is attenuated by catalase treatment	130
	Antioxidants prevent TERT nuclear export and downregulation of telomerase activity and delay replicative senescence of human ECs	134
	Aspirin prevents the increase in ROS formation and telomerase downregulation occurring during endothelial senescence	138
Cardiac myocytes	Overexpression of hTERT induces hypertrophy and inhibits apoptosis	94
	TRF2 inactivation causes telomere erosion, Chk2 activation, and increases apoptosis in cultured rat ventricular myocytes	95
	Short telomeres in late generation TERC-null mice upregulate the tumor suppressor protein p53 in cardiomyocytes	35

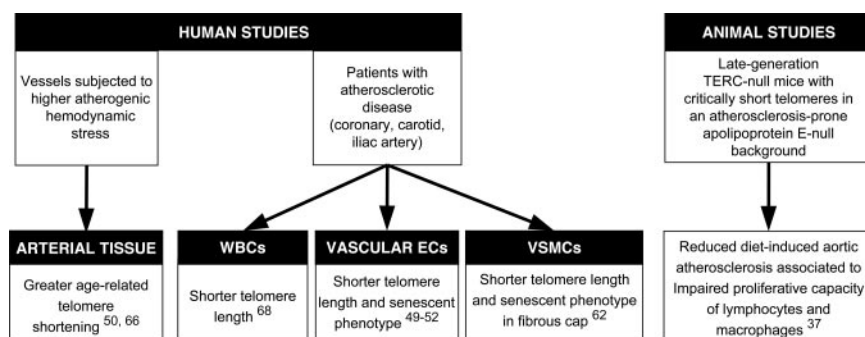
chromosome.<sup>26</sup> However, the influence of environmental factors on the rate of telomere attrition may also be of great importance in determining telomere length in adulthood. For example, whereas Okuda et al reported no differences in telomere length in several human tissues when comparing male and female newborns,<sup>21</sup> studies with humans and rodents have revealed higher telomerase activity<sup>27</sup> and longer telomeres<sup>8,9,28</sup> in females compared with males in various adult tissues. As discussed below, estrogen-dependent activation of telomerase may contribute to these gender-related differences in telomere length that arise during adulthood. Furthermore, prospective epidemiological studies have revealed higher telomere attrition rates in white blood cells (WBCs) of diabetic and obese human subjects<sup>29</sup> (see below).

The consequences of telomere ablation at the organismal level have been rigorously assessed in *TERC*-deficient mice,<sup>30–38</sup> which undergo progressive telomere shortening with each generation and lose viability when they reach critically short telomeres (typically after 3 to 5 generations). Remarkably, late generation *TERC*-null mice display premature aging symptoms and associated disorders, such as infertility, hair graying, alopecia, heart dysfunction, hypertension, various tissue atrophies and decreased tissue regen-

eration capacity.<sup>30–36,38</sup> These findings indicate that a minimal telomere length is required to maintain tissue homeostasis in the mouse and lend support to the notion that progressive telomere shortening may be involved in the pathogenesis of age-related human disorders. Accordingly, telomerase activity is impaired, or telomere attrition is accelerated, in various human premature aging syndromes, such as dyskeratosis congenita,<sup>39</sup> Werner syndrome,<sup>40</sup> or ataxia telangiectasia.<sup>41</sup> Remarkably, ectopic expression of telomerase rescues telomere defects in cells from patients experiencing dyskeratosis congenita thus supporting the importance of telomerase deficiency in the pathology of this disease.<sup>42</sup>

Because aging is a major cardiovascular risk factor,<sup>43,44</sup> addressing whether age-dependent telomere exhaustion affects cardiovascular pathobiology has been the center of intensive research in recent years. In the next sections, we discuss the results and main conclusions that have arisen from experimental and human studies that have linked telomere function to neovascularization and the development of atherosclerosis and heart disease, as well as to several factors known to influence cardiovascular risk, such as estrogens, oxidative stress, hypertension, diabetes, psychological stress, and smoking. We also discuss the potential use of “telomerase” strategies for cardiovascular therapy.





**Figure 3.** Associations between atherosclerosis and telomere exhaustion in different tissues and cell types from humans and TERC-null mice. Reference nos. are shown.

## Telomeres and Atherosclerosis

Atherosclerosis is a complex inflammatory process that involves both adaptive and innate immune mechanisms.<sup>43–48</sup> EC dysfunction triggered by atherogenic stimuli (eg, elevated plasma cholesterol level, hypertension, diabetes, and smoking) is of central importance in the pathogenesis of atherosclerosis. Table 1 summarizes the results of in vitro studies implicating telomeres and telomerase in EC function. In vivo, age-dependent telomere shortening has been reported in ECs from iliac, thoracic, and coronary arteries<sup>49–51</sup> (Figure 3). Minamino et al reported that vascular ECs with senescence-associated phenotypes are present in human atherosclerotic lesions.<sup>52</sup> Notably, a dominant-negative TRF2 mutant that destroys telomere structure<sup>53,54</sup> induces, when ectopically overexpressed in human ECs, a senescent phenotype as well as functional alterations involved in atherogenesis, such as increased expression of intercellular adhesion molecule 1 (ICAM-1) and diminished endothelial nitric oxide synthase (eNOS) activity.<sup>52</sup> Conversely, transduction of TERT inhibited both EC senescence and associated functional alterations.<sup>52</sup> Notably, Ogami et al found shorter telomeres in coronary ECs of patients with coronary artery disease (CAD) than in age-matched non-CAD patients, and in atherosclerotic coronary ECs compared with those at nonatherosclerotic sites in 6 individual CAD patients.<sup>51</sup> Collectively, these studies suggest that EC dysfunction and replicative senescence induced by telomere shortening play a critical role in coronary atherogenesis.

Once fatty streaks are formed, activated neointimal leukocytes produce a plethora of inflammatory mediators that contribute to atheroma growth by provoking excessive VSMC proliferation and migration.<sup>43,44,55–57</sup> Telomerase has been implicated as an important regulator of VSMC proliferation in vitro (Table 1), because TERT activation extends the lifespan of cultured VSMCs and, conversely, telomerase inhibition abrogates VSMC proliferation in a dose-dependent manner.<sup>58–60</sup> Regulation of VSMC proliferation by targeting telomerase activity appears to be independent of telomere length, because VSMC growth arrest occurs early after telomerase inhibition<sup>60,61</sup> and telomerase expression alone is capable of rescuing the senescent phenotype of human plaque VSMCs despite short telomeres.<sup>62</sup>

A role of telomerase on the control of VSMC growth has been also proposed in vivo. Telomerase activation and telomere maintenance appear to be critical for increased VSMC hyperplastic growth in hypertensive rats.<sup>63</sup> Further-

more, Ogawa et al found that telomerase activity and VSMC hyperplasia are concomitantly induced in murine femoral artery after endovascular artery denudation,<sup>61</sup> and Gupta et al reported a strong trend toward an association between telomerase activity and human restenosis,<sup>64</sup> which is also characterized by excessive VSMC proliferation.<sup>55,57</sup> Likewise, telomerase activity has been detected in 35% and 70% of human atherosclerotic coronary artery specimens retrieved by directional atherectomy<sup>64</sup> or obtained from heart transplant recipients,<sup>65</sup> respectively. Whereas hTERT immunoreactivity was faint or undetectable in 11 (85%) of 13 coronary arteries without morphological abnormalities (Stage 0/I/II/III), strong hTERT expression was detected in 14 (88%) of 16 coronary segments with atheromas grading IV to VI confined to the cell-rich area in the early thickened media/neointima or late advanced lesions with severe calcification and necrosis.<sup>65</sup> Notably, the frequency of hTERT immunoreactivity directly correlated with relative telomerase activity. In a recent study, Matthews et al found low levels of telomerase activity in plaque VSMCs and shorter telomeres in coronary plaques than in normal vessels from the same patients.<sup>62</sup> These authors identified fibrous cap VSMCs, which are a hallmark of advanced plaques, as the main neointimal cell type experiencing telomere attrition. Human plaque VSMCs within the fibrous cap exhibited a senescent phenotype and enhanced telomere loss compared with medial VSMCs or non-VSMCs of the same lesion. VSMC senescence was associated with changes in key cell cycle regulatory proteins (eg, cyclins D/E, p16, p21, and pRB), and telomerase expression alone rescued plaque VSMC senescence despite short telomeres, normalizing the changes in p16, p21, and pRB protein expression.

Based on the aforementioned observations, the emerging picture is that arterial cell hyperplastic growth at early stages of atherosclerosis may be facilitated by telomerase activation. Progressive telomere exhaustion in more advanced plaques, possibly caused by multiple population doublings in the presence of scarce or absent telomerase activity, may lead to the accumulation of senescent ECs and VSMCs. Possible factors causing telomerase inhibition and accelerated telomere shortening include oxidative stress, hypertension, and diabetes (see below). Enhanced EC turnover caused by hemodynamic stress may contribute to telomere ablation in this cell type, because age-dependent telomere shortening is more prominent in atherosclerosis-prone vessels subjected to high hemodynamic stress.<sup>50,66</sup>

EC dysfunction in atherosclerotic vessels promotes the adhesion and transendothelial migration of circulating leukocytes. Several studies have established an association between telomere length in WBCs and atherosclerosis. Patients with vascular dementia, a disorder that is frequently associated with cerebrovascular atherosclerosis and stroke, exhibit significantly shorter WBC telomeres compared with age-matched controls, namely cognitively competent patients experiencing from cerebrovascular or cardiovascular disease (CVD) alone, patients with probable Alzheimer's dementia, and apparently healthy individuals.<sup>67</sup> Likewise, after adjustment for age and sex, average telomere length in leukocytes of 10 patients with severe CAD (age 42 to 72 years) was significantly shorter compared with 20 controls with normal coronary angiograms (age 39 to 72 years).<sup>68</sup> Although analysis of 163 treated hypertensive men also revealed shorter WBC telomeres in subjects with carotid artery plaques versus subjects without plaques,<sup>69</sup> Kurz et al have reported that calcific aortic valve stenosis, but not CAD, is associated with shorter leukocyte telomeres in a cohort of 193 elderly patients (>70 years of age).<sup>70</sup>

Matsubara et al investigated whether the (−1327)T/C polymorphism within the hTERT gene affects promoter activity and leukocyte telomere length in normal individuals.<sup>71</sup> Activity of the hTERT promoter was significantly lower when the (−1327)C-sequence was present. Moreover, age-related telomere shortening was only clear in the (−1327)C-allele carriers, and telomeres were significantly longer in the (−1327)T-allele carriers. In a recent case-control study with 104 CAD patients and 115 age- and sex-matched controls, the same investigators found shorter WBC telomere length and a higher frequency of the hTERT (−1327)C/C genotype among cases compared with controls.<sup>72</sup>

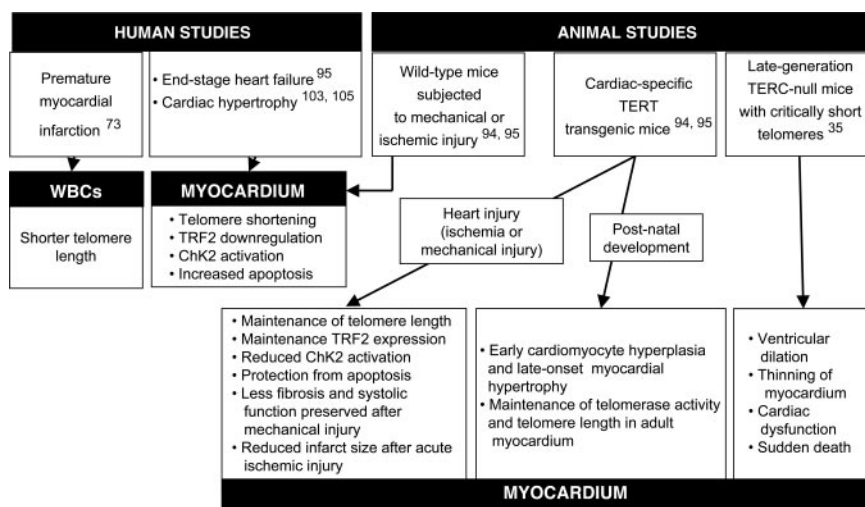
Myocardial infarction (MI) is frequently a complication of CAD patients. In a study comparing 203 cases with a premature MI (<50 years of age) and 180 controls, a significant association between shorter WBC telomere length and the risk of premature MI was detected after adjustment for age and sex.<sup>73</sup> Notably, subjects with shorter than average telomeres had between 2.8- and 3.2-fold higher risk of MI compared with subjects in the highest quartile for telomere length.<sup>73</sup> Moreover, analysis of 143 normal unrelated individuals more than 60 years of age revealed an association between shorter telomere length in blood DNA and poorer survival that was partly attributed to a 3.18-fold mortality rate from heart disease and a 8.54-fold higher mortality rate from infectious disease.<sup>74</sup> However, 2 recent studies suggest that WBC telomere length is not associated with morbidity or mortality in the oldest old.<sup>75,76</sup> First, although Bischoff et al observed in a cohort of 812 persons, aged 73 to 101 years, an association between longer telomeres in blood cells and better survival, no association was found when age was controlled for in the analyses.<sup>75</sup> Likewise, Martin-Ruiz et al concluded that WBC telomere length is not a predictive indicator for age-related morbidity and mortality at ages more than 85 years, possibly because of a high degree of telomere length instability in this group.<sup>76</sup>

Certainly, additional epidemiologic studies are needed to conclusively address if a link exists between leukocyte

telomere length and atherosclerosis in different vascular regions, MI, and survival. Thus, it cannot be concluded at present time that individuals with shorter telomeres might be at higher risk of developing atherosclerosis. Indeed, because excessive cell proliferation is a feature of atheroma development and the rate of telomere shortening augments in most somatic cells with increasing population doublings, an alternative explanation is that telomere ablation in WBCs and arterial tissue from patients with atherosclerosis is a mere consequence of increased cell turnover induced by the underlying chronic inflammatory response. To obtain further insight into this question, we investigated the impact of telomere exhaustion on diet-induced atherosclerosis in apolipoprotein E (apoE)-null mice, a well-established model of experimental atherosclerosis that recapitulates important aspects of the human disease.<sup>77</sup> We found that fourth generation mice doubly deficient in apoE and TERC (G4apoE-TERC-DKO) had shorter telomeres and were protected from aortic atherosclerosis compared with apoE-null mice with an intact TERC gene.<sup>37</sup> Remarkably, mitogen-induced proliferation was completely blunted in primary cultures of lymphocytes and macrophages obtained from G4apoE-TERC-DKO mice, suggesting that immunoreplicative senescence may be a mechanism by which telomere exhaustion protects from atheroma growth in this experimental model. It remains unresolved whether diminished VSMC proliferation may also contribute to reduced neointimal thickening in fat-fed G4apoE-TERC-DKO mice. Likewise, examination of appropriate animal models is needed to ascertain whether telomere attrition, by inducing VSMC senescence or apoptosis, may promote plaque rupture and thrombus formation.

Because human aging is associated with telomere erosion in most somatic cells,<sup>78</sup> the higher prevalence of atherosclerosis within the elderly appears to challenge the finding made in mice that short telomeres protect from atherosclerosis. Although profound differences in telomere biology between humans and rodents cannot be discarded, these seemingly conflicting findings might be reconciled if accepting that accumulation of cellular damage imposed by prolonged exposure to cardiovascular risk factors ultimately prevails over protective mechanisms, including telomere exhaustion. Consistent with this notion, diet-induced atherosclerosis was significantly reduced in old compared with young rabbits.<sup>79</sup> A definitive answer to the question of whether telomere ablation is a primary cause or a consequence of atherosclerosis requires prospective epidemiological studies to ascertain whether telomere length in newborns is a predictor of atherosclerosis and associated CVD in adulthood independently of well-established cardiovascular risk factors. In addition, large prospective epidemiological studies are needed to ascertain whether augmented rates of telomere attrition in WBCs are associated with atherosclerosis progression and the influence on this parameter of major atherogenic stimuli.

The limited replicative capacity of adult VSMCs may be a rate-limiting step in engineering blood vessels of clinical value for bypass surgery. Indeed, robust human vessels have been engineered *in vitro* using hTERT-expressing VSMCs, which proliferated far beyond their normal lifespan and



**Figure 4.** Associations among heart disease, telomerase, and telomere length in different tissues and cell types from humans and genetically modified mice. Reference nos. are shown.

retained phenotypic characteristics of normal VSMCs.<sup>58,80</sup> Apart from this potential use in vessel engineering for revascularization of ischemic tissues, it is still uncertain whether additional telomerization strategies may some day be of clinical application for patients with atherosclerotic disease. Indeed, a chief question is establishing whether telomere lengthening or exhaustion may be beneficial in these patients. On the one hand, maintaining/restoring telomere length could prevent or ameliorate endothelial senescence/dysfunction and improve neovascularization of ischemic territories (see next section). However, long telomeres may accelerate atheroma progression by exacerbating neointimal leukocyte and VSMC proliferation. Of note in this regard, reduced TERT activity appears required for the antiproliferative effects that thiazolidinediones exert on VSMCs,<sup>61</sup> although it is unknown whether TERT inhibition by these peroxisome proliferator-activated receptor- $\gamma$  ligands used as antidiabetic drugs in patients with type 2 diabetes is also necessary for their antirestenotic effects in the vasculature.<sup>81</sup>

### Telomeres and Neovascularization

The restoration of blood flow into ischemic regions in the adult organism depends on *de novo* vessel formation by endothelial progenitor cells (EPCs) (vasculogenesis) and on the development of new collateral vessels from established vascular networks (angiogenesis).<sup>82</sup> On the other hand, an important body of evidence implicates neointimal angiogenesis as a mechanism contributing to atheroma growth.<sup>83,84</sup>

TERC-null mice with critically short telomeres display a marked reduction in angiogenesis in both Matrigel implants and murine melanoma grafts, which was associated with reduced and increased tumor cell proliferation and apoptosis, respectively, as well as a lower tumor growth rate.<sup>34</sup> Thus, it is tempting to speculate that telomere exhaustion may contribute to age-dependent impairment of angiogenesis.<sup>85</sup> Further evidence implicating telomerase as a key regulator of neovascularization is provided by the observation that hTERT mRNA expression in ECs of newly formed vessels directly correlates with the histological grade of human tumors.<sup>86</sup> It will be of great interest to identify the diffusible factor(s) produced by glioblastoma cells *in vitro* that upregulate hTERT mRNA and protein expression and telomerase

activity in ECs.<sup>87</sup> The demonstration that hypoxia, a fundamental angiogenic stimulus, induces in VSMCs TERT protein expression and phosphorylation<sup>59</sup> also highlights the importance of telomerase for angiogenesis. Notably, overexpression of hTERT in human dermal microvascular ECs augments their capacity to form more durable microvascular structures when subcutaneously xenografted in severe combined immunodeficiency mice.<sup>88</sup> Likewise, hTERT gene transfer improves the proliferative and migratory activity as well as the survival of human EPCs and increases neovascularization and limb salvage in a murine hindlimb ischemia model.<sup>89</sup> However, it is important to note that telomere-independent barriers might limit the transplantation potential of murine hematopoietic stem cells, because overexpression of TERT in these cells did not extend transplantation capacity in spite of preserving telomere loss during serial transplantation.<sup>90</sup>

### Telomeres and Heart Pathobiology

As in other adult tissues, telomerase activity in the adult heart is markedly downregulated after birth and remains low or absent through adulthood, both in rodents<sup>91–94</sup> and in humans.<sup>14,16,95</sup> It is widely accepted that most cardiac cells are differentiated postmitotic myocytes, and it has been postulated that restoring their proliferative capacity by increasing telomerase activity may facilitate the regeneration of myocardial tissue after MI or the restitution of chronic myocyte loss in heart failure. Indeed, transgenic mice engineered to express TERT specifically in cardiomyocytes maintain telomerase activity and telomere length in the adult heart, and this correlated with reduced area of MI, less fibrous area, and preservation of systolic function after mechanical and ischemic injury (Figure 4).<sup>94,95</sup> It is noteworthy that cardiac TERT expression induced a biphasic response with decreased myocyte size and increased cell density in the heart of 2-week-old transgenic mice, and ventricular hypertrophy and increased myocyte size at 12 weeks of age. Thus, cardiac-specific TERT expression in the mouse may initially delay cardiac myocyte cell cycle exit and subsequently induce late-onset cell hypertrophy. However, primary cultures of postmitotic



rat ventricular myocytes overexpressing TERT exhibited hypertrophic growth but did not undergo DNA replication.<sup>94</sup>

Leri et al detected telomerase activity in pure preparations of young adult, fully mature adult, and senescent rat ventricular myocytes,<sup>27</sup> in contrast to previous studies reporting lack of telomerase activity in the adult heart.<sup>92</sup> Because telomerase activity is a characteristic of proliferating cells, these findings were interpreted as an indication that replicative-competent cardiac cells exist throughout life and that these cells may counteract the continuous cell death in the aging mammalian heart. Indeed, defeating the dogma that adult heart is a terminally differentiated organ without self-renewal potential, several reports have demonstrated the existence of a subpopulation of replicating cardiomyocytes in normal and pathological states.<sup>96–100</sup> Moreover, increasing evidence supports the existence of telomerase-expressing multipotent cardiac stem cells (CSCs), which contribute to myocardial regeneration in the adult heart.<sup>101–104</sup>

Table 1 summarizes the results of *in vitro* studies implicating telomeres and telomerase in cardiomyocyte function. Several *in vivo* studies have investigated the role of telomerase and telomere length in the pathogenesis of heart disease (Figure 4). As discussed above, reduced telomere length in WBCs is associated with increased risk of MI and heart disease and higher mortality rate.<sup>73,74</sup> Telomere attrition in the heart has also been detected in patients experiencing from age-related cardiomyopathy (characterized by an increase of cell senescence markers, moderate hypertrophy and cardiac dilatation)<sup>105</sup> and in patients with cardiac hypertrophy consecutive to aortic stenosis with a mean duration of 3 years,<sup>103</sup> in spite of increased telomerase activity in the heart in both cases. In contrast, in a dog model of acute heart failure with progressive deterioration of cardiac performance and dilated cardiomyopathy, telomerase activity increased during the onset and progression of ventricular dysfunction and, in this case, appeared sufficient to preserve telomere length.<sup>106</sup> Notably, it has been suggested that telomere shortening impairs CSC-mediated myocardial regeneration by limiting the proliferative potential of these cells. Supporting this notion, Urbanek et al found that telomerase-competent CSCs increase in number immediately after MI, but this growth response is attenuated in chronic heart failure concomitant with decreased telomerase activity, severe telomeric shortening, and the accumulation of senescent cells.<sup>104</sup>

The examination of successive generations of TERC-null mice additionally highlights the importance of telomere attrition in cardiac pathology (Figure 4).<sup>35</sup> Aged fifth-generation TERC-deficient mice (G5TERC-KO) exhibited significantly shorter telomeres in cardiomyocytes, ventricular dilation, thinning of the myocardium, cardiac dysfunction, and sudden death. Heart sections from G5TERC-KO mice revealed increased level of expression of the tumor suppressor protein p53, reduced proliferation, and increased apoptosis, as well as a 50% reduction in the number of left ventricular myocytes compared with wild-type mice. Notably, p53 protein level and telomere exhaustion were correlated in G5TERC-KO cardiomyocytes. The pathways connecting telomeres and p53 in senescence and apoptosis are reviewed elsewhere.<sup>107</sup>

Apart from its possible role in establishing replicative senescence of CSCs, telomere dysfunction may also contribute to heart disease by inducing apoptosis. Increased cell death, frequently associated to decreased levels of the telomere-associated protein TRF2, has been detected in senescent cardiac cells with significant telomere shortening in aged diseased hearts<sup>105</sup> and in hearts of patients experiencing from acute and chronic MI<sup>104</sup> or heart failure.<sup>95</sup> Remarkably, apoptosis after ischemic (coronary ligation) and biomechanical (partial occlusion of the thoracic aorta) injury is attenuated in the heart of transgenic mice with cardiac-specific TERT overexpression, and this correlated with reduced area of MI, less fibrous area, and preservation of systolic function.<sup>94,95</sup>

Based on the observations made in G5TERC-KO<sup>35</sup> and cardiac-specific TERT transgenic mice,<sup>94</sup> and the demonstration that critically short telomeres in late-generation TERC-null mice can become fully functional by telomerase restoration,<sup>108</sup> telomerase gene transfer may open new opportunities for myocardial repair. However, potentially harmful effects associated to telomerization must be considered, including the following: (1) accelerated progression of atherosclerotic plaques in CAD patients caused by homing of telomerized cells into coronary vessels, because neovascularization is enhanced by TERT overexpression<sup>88,89</sup> and proliferation of vasa vasorum promotes atherosclerosis<sup>83,84</sup>; and (2) development of cardiac fibrosis and cancer as a result of indiscriminate proliferation of telomerized cells within the heart.<sup>109,110</sup> These potential risks could be attenuated by grafting cells engineered to conditionally overexpress TERT in a temporal and cardiac-restricted manner. An alternative approach may be the use of telomerase-competent CSCs, which can be isolated and *ex vivo* expanded and are capable of homing to the injured myocardium of experimental animals when injected either intravenously<sup>102</sup> or directly into the ischemic heart.<sup>101</sup>

### Telomeres and Factors Affecting Cardiovascular Risk

This section discusses *in vitro* and *in vivo* findings that have established links between telomere homeostasis and factors influencing CVD development (Table 2).

#### Estrogens

The lower incidence of CVD in premenopausal women compared with men may be attributable, at least in part, to estrogens.<sup>111–114</sup> In addition to the well-characterized actions on lipoprotein metabolism and on vascular cells, the influence of estrogen on telomere homeostasis may also contribute to their beneficial effects on the cardiovascular system. Both human and animal studies have revealed higher telomerase activity and diminished rate of age-related telomere ablation resulting in longer telomeres in females than in males.<sup>8,9,24,26–28</sup> Estrogen may indirectly exert a protective effect on telomeres because of its antioxidant properties.<sup>115</sup> Moreover, evidences for direct effects of estrogens on telomerase activity have been provided both *in vitro* and *in vivo*. First, telomerase activity in the endometrium varies across the menstrual cycle.<sup>116</sup> Endometrial telomerase activity is negligible during



**TABLE 2. Human Studies Reporting Associations Between Factors Affecting Cardiovascular Risk and Telomere Length or Telomerase Activity**

Cardiovascular Risk Modulator	Main Findings	References
Gender	Women display higher telomeres compared with men in WBCs from adult individuals	24, 26, 28
Oxidative stress	Systemic oxidative stress is associated with shorter WBC telomere length in hypertensive men	132
	Oxidative damage correlates negatively with monocyte telomere length in type 2 diabetic patients	142
Hypertension	WBC telomere length is inversely correlated with pulse pressure and hypertension	24, 28
Type 1 diabetes mellitus	WBCs of patients with type 1 diabetes have significantly shorter telomeres than those of nondiabetic control subjects	140
Type 2 diabetes mellitus, insulin resistance, and obesity	Leukocytes from type 2 diabetic patients have significantly shorter telomeres than those of nondiabetic subjects	141, 142
	Insulin resistance and obesity correlate with enhanced telomere attrition rate in WBCs	29
	Insulin resistance is inversely correlated with leukocyte telomere length	132
	Obesity is inversely correlated with WBC telomere length in women	147
Smoking	WBCs from smoking women have shorter telomere length	147
	Sex- and age-adjusted WBC telomere length is lower in smokers than in nonsmokers	26
Psychological stress	Psychological stress is significantly associated to higher oxidative stress, lower telomerase activity, and shorter telomere length in WBCs	145

menstruation but increases during the follicular phase, reaches maximum levels immediately before ovulation, coinciding with a peak in estrogen levels and proliferative activity, and then falls during the luteal phase. Of note, postmenopausal endometrium and endometrium from women treated with antiestrogens exhibit decreased telomerase activity. Second, estrogens induce TERT transcription in MCF-7 cells via an estrogen response element within the TERT promoter,<sup>117</sup> and activate in human ECs the phosphatidylinositol 3-kinase/Akt pathway,<sup>118</sup> which in turn enhances human telomerase activity through hTERT phosphorylation.<sup>119</sup> Raloxifene, a selective estrogen receptor modulator, also upregulates telomerase activity in human umbilical ECs (HUVECs).<sup>120</sup> In accordance with these findings, Imanishi et al found that estrogen reduces EPC senescence by increasing TERT levels in a phosphatidylinositol 3-kinase/Akt-dependent manner.<sup>121</sup> Furthermore, estrogen stimulates in vascular ECs the production of nitric oxide,<sup>118</sup> which also induces telomerase activity and delays EC senescence.<sup>122</sup>

### Oxidative Stress

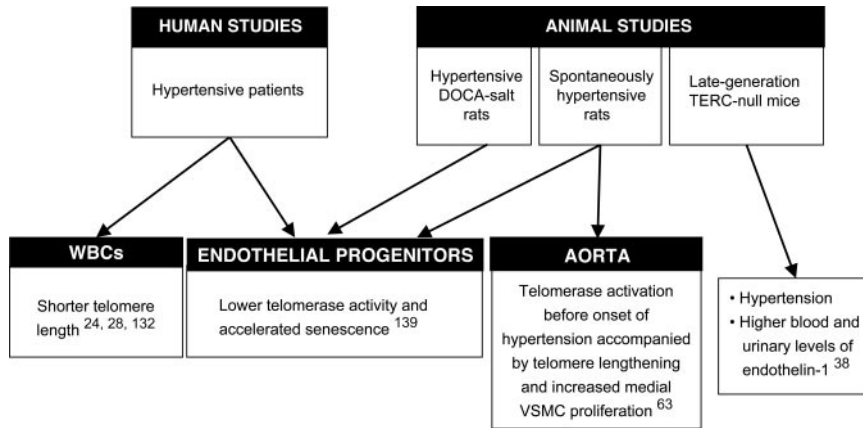
Accumulation of oxidative damage is thought to play an important role in aging and associated diseases,<sup>123</sup> including CVD.<sup>124,125</sup> Reactive oxygen species (ROS) regulate endothelium-dependent vasodilation and VSMC growth, differentiation, and apoptosis. Furthermore, lipid peroxidation and protein nitration induced by some ROS are early events in the atherosclerotic process. In recent years, an increasing body of evidence has implicated oxidative damage in telomere dysfunction in vascular cells. Hydrogen peroxide and oxidized low-density lipoproteins inhibit EC telomerase activity.<sup>126</sup> Similarly, long-term exposure of HUVECs to mild oxidative stress induced by perturbation of the glutathione redox cycle resulted in accelerated downregulation of telomerase activity, enhanced telomere erosion, and the premature onset of replicative senescence.<sup>127</sup> Consistent with these findings,

maximum levels of glutathione in proliferating 3T3 fibroblasts coincided with a peak of telomerase activity, and glutathione depletion and restoration decreased and restored, respectively, telomerase activity in these cells.<sup>128</sup> Homocysteine, a cardiovascular risk factor whose atherogenic effects have been ascribed to increased hydrogen peroxide production,<sup>129</sup> also increased the rate of telomere shortening in ECs, and this effect was attenuated in a dose-dependent manner by catalase treatment.<sup>130</sup> On the other hand, prolonged oxidative damage also inhibited telomerase activity and accelerated telomere shortening in VSMCs,<sup>62</sup> an effect that may be mediated by the formation of 8-oxodG at the GGG triplet in telomeric DNA.<sup>131</sup> Recently, Demissie et al reported that systemic oxidative stress assessed by urinary 8-epi-prostaglandin F<sub>2α</sub> is associated with shorter WBC telomere length in hypertensive men from the Framingham Heart Study.<sup>132</sup>

The aforementioned studies are consistent with the notion that oxidative stress inhibits telomerase activity and promotes telomere exhaustion. Consistent with this view, several antioxidants attenuate telomere shortening. ROS scavenging by Asc2P, an oxidation-resistant derivative of vitamin C, slows down telomere shortening and expands the lifespan of HUVECs.<sup>133</sup> Likewise, Haendeler et al<sup>134</sup> demonstrated that reduction of intracellular reactive oxygen species formation by treatment of ECs with the antioxidant *N*-acetylcysteine delays the loss of telomerase activity and the onset of age-related replicative senescence, and inhibits the nuclear export of TERT protein, a mechanism by which oxidative stress may inhibit telomerase activity via Src kinase family-dependent phosphorylation of hTERT at tyrosine 707.<sup>135</sup> Aspirin, which possesses antioxidant properties,<sup>136,137</sup> also prevents the increase in ROS formation and telomerase downregulation occurring during endothelial senescence.<sup>138</sup>

### Hypertension

A major factor contributing to CVD in the elderly is hypertension.<sup>46</sup> Figure 5 summarizes animal and human studies that



**Figure 5.** Associations among hypertension, telomerase, and telomere length in different tissues and cell types from humans and experimental animals. Reference nos. are shown.

have linked telomerase and telomere alterations to hypertension. Cao et al reported that both TERT expression and telomerase activity are induced in the aorta, but not in other tissues, of spontaneously hypertensive rats (SHR).<sup>63</sup> These changes occurred before the onset of hypertension, and were accompanied by telomere lengthening and increased proliferation of medial VSMCs. TERT downregulation by antisense RNA inhibited proliferation and increased apoptosis of cultured VSMCs, a response that was abrogated by ectopically overexpressed p53. Although these findings suggest that early telomerase activation in aortic medial VSMCs may contribute to the initial phases of vascular remodeling associated to hypertension, Perez-Rivero et al recently found that TERC-deficient mice develop hypertension.<sup>38</sup> This phenotype was associated with higher blood and urinary levels of the endothelium-derived vasoconstrictor peptide endothelin-1 (ET-1). Because no differences in the expression of the precursor pre-pro-ET-1 were detected in aorta and renal cortex of TERC-null mice, Perez-Rivero et al postulated that increased levels of circulating ET-1 may be attributable to increased expression of the endothelin-converting enzyme (ECE-1), which converts pre-pro-ET-1 into biologically active ET-1. In fact, ECE-1 mRNA expression was significantly higher in TERC-deficient mice than in wild-type counterparts, and ECE-1 promoter activity was increased in TERC-deficient murine embryonic fibroblasts (MEFs). These cells also displayed enhanced production of ROS and their treatment with antioxidants, such as catalase and *N*-acetylcysteine, reduced ECE-1 promoter activity, thus suggesting a causal link between ROS synthesis and ET-1 levels. Given these findings, the authors hypothesized that telomerase deficiency and telomere shortening may modify the phenotypic characteristics of vascular cells in a way that favors development of hypertension (eg, increasing ET-1 production). Accordingly, EPCs from hypertensive patients and from SHR and deoxycorticosterone acetate (DOCA)-salt rats undergo accelerated senescence concomitantly with lowered telomerase activity.<sup>139</sup>

Several studies have examined the potential relationship between telomere length and human hypertension. By analyzing WBC telomere length in 49 twin pairs that included 38 men and 60 women 18 to 44 years of age, Jeanclos et al found that telomere restriction fragment length correlated negatively with systolic blood pressure but positively with dia-

stolic blood pressure, thus suggesting a negative relation between telomere length and pulse pressure.<sup>24</sup> Both pulse pressure and telomere length appeared highly heritable and the correlation between these parameters was sex independent. In another study that included 120 men and 73 women (mean age:  $56 \pm 11$  years) who were not taking any antihypertensive medication, Benetos et al found a negative correlation between telomere length and age in both sexes, but telomere shortening significantly contributed to increased pulse pressure and pulse wave velocity only in men.<sup>28</sup> Both studies found age-adjusted longer telomeres in women, possibly as a consequence of the beneficial effects of estrogens on telomere homeostasis (see above). More recently, Demissie et al confirmed the association between shorter leukocyte telomere length and hypertension in 327 men from the Framingham Heart study and suggested that increased telomere attrition in hypertensives was largely attributable to insulin resistance.<sup>132</sup>

### Diabetes

Diabetic patients are at higher risk for microvascular and macrovascular disease.<sup>45</sup> Jeanclos et al reported that telomere length in WBCs from patients with type 1 diabetes, an autoimmune disease in which subsets of immune cells are involved in the destruction of pancreatic  $\beta$  cells, is reduced compared with age-matched nondiabetic controls.<sup>140</sup> Because this parameter was undistinguishable when comparing type 2 diabetic patients and nondiabetic controls, the authors suggested that telomere shortening occurs in subsets of WBCs that play a role in the pathogenesis of type 1 diabetes. However, recent reports have shown that shorter telomeres are also associated to insulin resistance and to type 2 diabetes.<sup>29,132,141–143</sup> Notably, Gardner et al found that insulin resistance and obesity correlate with an enhanced rate of telomere attrition, which may be caused by heightened oxidative stress or inflammation occurring in these conditions.<sup>29</sup>

### Psychological Stress and Smoking

It is generally accepted that psychological stress leads to accelerated aging and the premature onset of age-related disease. Chronic stress has been linked to increased cardiovascular risk,<sup>144</sup> although the underlying mechanisms remain

largely unknown. Of note in this regard, chronic psychological stress correlated with higher oxidative stress and shorter telomere length in WBCs from healthy premenopausal women.<sup>145</sup>

Cigarette smoking is another important cardiovascular risk factor related to augmented oxidative stress.<sup>146</sup> By examining a random sample of Belgian families from the cohort of the Flemish Study on Environment, Genes and Health Outcomes, Nawrot et al found shorter telomeres in WBCs from smokers compared with nonsmokers after adjusting for sex and age.<sup>26</sup> More recently, Valdes et al confirmed that WBCs from smoking women have shorter telomeres and suggested that smoking reduces WBC telomere length in a dose-dependent manner.<sup>147</sup> However, it has been reported that a history of smoking, assessed either in terms of smoking status or pack years, has no independent effects on mean telomere restriction fragment length in WBCs<sup>73</sup> and that WBC telomere attrition is not correlated with smoking in individuals  $\geq 70$  years of age.<sup>70,75</sup>

### Conclusions and Future Perspectives

The following findings have conclusively linked telomere biology and CVD: (1) Like in other cell types, gain- and loss-of-function experiments demonstrate that telomerase and functional telomeres are necessary for maintaining the proliferative potential and viability of cultured ECs, VSMCs and cardiomyocytes; (2) short telomeres have been detected in senescent ECs and VSMCs from human atherosclerotic plaque, in myocardial tissue from patients with end-stage heart failure and cardiac hypertrophy, as well as in WBCs from patients with CAD, premature MI, hypertension, and diabetes mellitus, although some controversy among different epidemiologic studies exist; (3) TERC-null mice with critically short telomeres develop hypertension, ventricular dilation, thinning of myocardium, cardiac dysfunction, and sudden death; in contrast, heart damage induced by mechanical injury and ischemia is reduced in transgenic mice with cardiac-specific expression of TERT, which maintain telomere length in adult myocardium.

A chief question that remains unanswered is whether telomere exhaustion is cause or consequence of CVD. Given that an accelerated rate of telomere shortening may be expected from the increased cellular turnover associated to inflammation occurring in atherosclerosis, and from the action of several cardiovascular risk factors (eg, oxidative stress, hypertension, diabetes, smoking, psychological stress), telomere exhaustion may be a surrogate marker of CVD. However, it is tempting to speculate that short telomere length at birth, which seems to be mostly genetically determined, predisposes to atherosclerosis and associated CVD, in part as a result of premature senescence of leukocytes, vascular cells, and cardiac myocytes. Contrary to this notion, hypercholesterolemic mice doubly deficient in apoE and TERC had shorter telomeres, exhibited a marked reduction in leukocyte proliferation, and were protected from atherosclerosis compared with apoE-null mice with an intact TERC gene. Thus, although telomerase strategies aimed at maintaining/restoring telomere length may prevent or ameliorate endothelial senescence/

dysfunction and improve neovascularization of ischemic territories, such an approach may accelerate atheroma progression by aggravating neointimal leukocyte and VSMC proliferation. On the other hand, it remains unexplored whether alterations in telomere length affect thrombus formation, a frequent cause of MI and stroke in patients with atherosclerotic disease.

Another field of interest is the possible application of telomerase in tissue engineering, for instance in the generation of artificial blood vessels and in cell-based myocardial regeneration. The use of autologous donor cells for these purposes is often not viable because of their limited replicative lifespan, particularly when they are obtained from elderly patients, an obstacle that may be circumvented by telomerase gene transfer. However, concern exists regarding the safety of this approach. Specifically, telomerization may aggravate atherosclerosis by promoting vessel neovascularization. Moreover, because telomerase endow normal cells with unlimited proliferative potential and is reactivated in most human tumors, indiscriminate telomere lengthening within the heart may provoke fibrosis and cancer. Of note in this regard, hTERT overexpression in VSMCs from elderly patients does not lead to a transformed phenotype at population doubling  $\leq 100$ .<sup>148</sup> The potential side effects of telomerase gene transfer could be limited by either using donor cells with conditional hTERT overexpression or telomerase-competent CSCs.

In conclusion, more basic research and large epidemiological studies are needed to conclusively ascertain whether telomere erosion is an independent cardiovascular risk factor or a consequence of CVD and to examine the efficacy of novel therapeutic strategies aimed at modifying telomere length. The generation of genetically engineered mice exhibiting tissue-specific alterations in telomerase and additional telomere-associated proteins with possible roles in the pathogenesis of CVD remains unexplored and would be an invaluable tool to answer these questions.

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### Disclosures

None.

### References

1. Blackburn EH. Switching and signaling at the telomere. *Cell* 2001;106:661–673.
2. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet*. 2005;6:611–622.
3. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science*. 1994;266:2011–2015.



4. Counter CM, Botelho FM, Wang P, Harley CB, Bacchetti S. Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes. *J Virol*. 1994;68:3410–3414.
5. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc Natl Acad Sci U S A*. 1995;92:4818–4822.
6. Gollahon LS, Kraus E, Wu TA, Yim SO, Strong LC, Shay JW, Tainsky MA. Telomerase activity during spontaneous immortalization of Li-Fraumeni syndrome skin fibroblasts. *Oncogene*. 1998;17:709–717.
7. Geserick C, Blasco MA. Novel roles for telomerase in aging. *Mech Ageing Dev*. 2006;127:579–583.
8. Coviello-McLaughlin GM, Prowse KR. Telomere length regulation during postnatal development and ageing in *Mus spretus*. *Nucleic Acids Res*. 1997;25:3051–3058.
9. Cherif H, Tarry JL, Ozanne SE, Hales CN. Ageing and telomeres: a study into organ- and gender-specific telomere shortening. *Nucleic Acids Res*. 2003;31:1576–1583.
10. Hiyaama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyaama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol*. 1995;155:3711–3715.
11. Chiu CP, Dragowska W, Kim NW, Vaziri H, Yui J, Thomas TE, Harley CB, Lansdorf PM. Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow. *Stem Cells*. 1996;14:239–248.
12. Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Weng NP. Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. *Proc Natl Acad Sci U S A*. 1999;96:5147–5152.
13. Blasco MA, Funk W, Villeponteau B, Greider CW. Functional characterization and developmental regulation of mouse telomerase RNA. *Science*. 1995;269:1267–1270.
14. Ulaner GA, Giudice LC. Developmental regulation of telomerase activity in human fetal tissues during gestation. *Mol Hum Reprod*. 1997;3:769–773.
15. Martín-Rivera L, Herrera E, Albar JP, Blasco MA. Expression of mouse telomerase catalytic subunit in embryos and adult tissues. *Proc Natl Acad Sci U S A*. 1998;95:10471–10476.
16. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR. Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. *Cancer Res*. 1998;58:4168–4172.
17. Yi X, White DM, Aisner DL, Baur JA, Wright WE, Shay JW. An alternate splicing variant of the human telomerase catalytic subunit inhibits telomerase activity. *Neoplasia*. 2000;2:433–440.
18. Colgin LM, Wilkinson C, Englezou A, Kilian A, Robinson MO, Reddel RR. The hTERT $\alpha$  splice variant is a dominant negative inhibitor of telomerase activity. *Neoplasia*. 2000;2:426–432.
19. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR. Tissue-specific alternate splicing of human telomerase reverse transcriptase (hTERT) influences telomere lengths during human development. *Int J Cancer*. 2001;91:644–649.
20. Saeboe-Larsen S, Fossberg E, Gaudernack G. Characterization of novel alternative splicing sites in human telomerase reverse transcriptase (hTERT): analysis of expression and mutual correlation in mRNA isoforms from normal and tumour tissues. *BMC Mol Biol*. 2006;7:26.
21. Okuda K, Bardeguet A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. *Pediatr Res*. 2002;52:377–381.
22. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet*. 1994;55:876–882.
23. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev*. 2000;119:89–99.
24. Jeanclos E, Schork NJ, Kyvik KO, Kimura M, Skurnick JH, Aviv A. Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension*. 2000;36:195–200.
25. Takubo K, Izumiyama-Shimomura N, Honma N, Sawabe M, Arai T, Kato M, Oshimura M, Nakamura K. Telomere lengths are characteristic in each human individual. *Exp Gerontol*. 2002;37:523–531.
26. Nawrot TS, Staessen JA, Gardner JP, Aviv A. Telomere length and possible link to X chromosome. *Lancet*. 2004;363:507–510.
27. Leri A, Malhotra A, Liew CC, Kajstura J, Anversa P. Telomerase activity in rat cardiac myocytes is age and gender dependent. *J Mol Cell Cardiol*. 2000;32:385–390.
28. Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*. 2001;37:381–385.
29. Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation*. 2005;111:2171–2177.
30. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorf PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*. 1997;91:25–34.
31. Lee HW, Blasco MA, Gottlieb GJ, Horner JW 2nd, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. *Nature*. 1998;392:569–574.
32. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell*. 1999;96:701–712.
33. Herrera E, Samper E, Martín-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. *EMBO J*. 1999;18:2950–2960.
34. Franco S, Segura I, Riese HH, Blasco MA. Decreased B16F10 melanoma growth and impaired vascularization in telomerase-deficient mice with critically short telomeres. *Cancer Res*. 2002;62:552–559.
35. Leri A, Franco S, Zacheo A, Barlucchi L, Chimenti S, Limana F, Nadal-Ginard B, Kajstura J, Anversa P, Blasco MA. Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J*. 2003;22:131–139.
36. Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, Gu Y, Alt FW, DePinho RA. Telomere dysfunction and ATM deficiency compromises organ homeostasis and accelerates ageing. *Nature*. 2003;421:643–648.
37. Poch E, Carbonell P, Franco S, Díez-Juan A, Blasco MA, Andrés V. Short telomeres protect from diet-induced atherosclerosis in apolipoprotein E-null mice. *FASEB J*. 2004;18:418–420.
38. Perez-Rivero G, Ruiz-Torres MP, Rivas-Elena JV, Jerkic M, Díez-Marques ML, Lopez-Novoa JM, Blasco MA, Rodríguez-Puyol D. Mice deficient in telomerase activity develop hypertension because of an excess of endothelin production. *Circulation*. 2006;114:309–317.
39. Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature*. 2001;413:432–435.
40. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, DePinho RA. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet*. 2004;36:877–882.
41. Metcalfe JA, Parkhill J, Campbell L, Stacey M, Biggs P, Byrd PJ, Taylor AMR. Accelerated telomere shortening in ataxia telangiectasia. *Nat Genet*. 1996;13:350–353.
42. Wong JM, Collins K. Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. *Genes Dev*. 2006;20:2848–2858.
43. Lusis AJ. Atherosclerosis. *Nature*. 2000;407:233–241.
44. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
45. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA*. 2002;287:2570–2581.
46. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335:765–774.
47. Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, Witztum JL. Innate and acquired immunity in atherogenesis. *Nat Med*. 2002;8:1218–1226.
48. Greaves DR, Channon KM. Inflammation and immune responses in atherosclerosis. *Trends Immunol*. 2002;23:535–541.
49. Aviv H, Khan MY, Skurnick J, Okuda K, Kimura M, Gardner J, Priolo L, Aviv A. Age dependent aneuploidy and telomere length of the human vascular endothelium. *Atherosclerosis*. 2001;159:281–287.
50. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci U S A*. 1995;92:11190–11194.
51. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, Hai E, Shirai N, Ehara S, Komatsu R, Naruko T, Ueda M. Telomere shortening



- in human coronary artery diseases. *Arterioscler Thromb Vasc Biol.* 2004;24:546–550.
52. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis. Role of telomere in endothelial dysfunction. *Circulation.* 2002;105:1541–1544.
  53. van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. *Cell.* 1998;92:401–413.
  54. Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science.* 1999;283:1321–1325.
  55. Rivard A, Andrés V. Vascular smooth muscle cell proliferation in the pathogenesis of atherosclerotic cardiovascular diseases. *Histol Histopathol.* 2000;15:557–571.
  56. Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med.* 2002;8:1249–1256.
  57. Andrés V. Control of vascular cell proliferation and migration by cyclin-dependent kinase signalling: new perspectives and therapeutic potential. *Cardiovasc Res.* 2004;63:11–21.
  58. McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, Niklason LE, Counter CM. Human arteries engineered in vitro. *EMBO Rep.* 2003;4:633–638.
  59. Minamino T, Mitsialis SA, Kourembanas S. Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. *Mol Cell Biol.* 2001;21:3336–3342.
  60. Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circ Res.* 2001;89:237–243.
  61. Ogawa D, Nomiya T, Nakamachi T, Heywood EB, Stone JF, Berger JP, Law RE, Bruemmer D. Activation of peroxisome proliferator-activated receptor gamma suppresses telomerase activity in vascular smooth muscle cells. *Circ Res.* 2006;98:e50–59.
  62. Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res.* 2006;99:156–164.
  63. Cao Y, Li H, Mu F-T, Ebisui O, Funder JW, Liu J-P. Telomerase activation causes vascular smooth muscle cell proliferation in genetic hypertension. *FASEB J.* 2002;16:96–98.
  64. Gupta M, Shogreen MR, Braden GA, White WL, Sane DC. Prevalence of telomerase in coronary artery atherosclerosis. *J Antiaging Med.* 2000;3:15–24.
  65. Liu S-C, Wang S-S, Wu M-Z, Wu D-C, Yu F-J, Chen W-J, Chiang F-T, Yu M-F. Activation of telomerase and expression of human telomerase reverse transcriptase in coronary atherosclerosis. *Cardiovasc Pathol.* 2005;14:232–240.
  66. Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, Aviv A. Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. *Atherosclerosis.* 2000;152:391–398.
  67. von Zglinicki T, Serra V, Lorenz M, Saretzki G, Lenzen-Grossimlghaus R, Gessner R, Risch A, Steinhagen-Thiessen E. Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab Invest.* 2000;80:1739–1747.
  68. Samani NJ, Boulton R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. *Lancet.* 2001;358:472–473.
  69. Benetos A, Gardner JP, Zureik M, Labat C, Xiaobin L, Adamopoulos C, Temmar M, Bean KE, Thomas F, Aviv A. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. *Hypertension.* 2004;43:182–185.
  70. Kurz DJ, Kloekener-Gruissem B, Akhmedov A, Eberli FR, Buhler I, Berger W, Bertel O, Luscher TF. Degenerative aortic valve stenosis, but not coronary disease, is associated with shorter telomere length in the elderly. *Arterioscler Thromb Vasc Biol.* 2006;26:e114–e117.
  71. Matsubara Y, Murata M, Yoshida T, Watanabe K, Saito I, Miyaki K, Omae K, Ikeda Y. Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT. *Biochem Biophys Res Commun.* 2006;341:128–131.
  72. Matsubara Y, Murata M, Watanabe K, Saito I, Miyaki K, Omae K, Ishikawa M, Matsushita K, Iwanaga S, Ogawa S, Ikeda Y. Coronary artery disease and a functional polymorphism of hTERT. *Biochem Biophys Res Commun.* 2006;348:669–672.
  73. Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol.* 2003;23:842–846.
  74. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet.* 2003;361:393–395.
  75. Bischoff C, Petersen HC, Graakjaer J, Andersen-Ranberg K, Vaupel JW, Bohr VA, Kolvraa S, Christensen K. No association between telomere length and survival among the elderly and oldest old. *Epidemiology.* 2006;17:190–194.
  76. Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, Westendorp RG. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell.* 2005;4:287–290.
  77. Breslow JL. Mouse models of atherosclerosis. *Science.* 1996;272:685–688.
  78. Collins K, Mitchell JR. Telomerase in the human organism. *Oncogene.* 2002;21:564–579.
  79. Cortés MJ, Díez-Juan A, Pérez P, Pérez-Roger I, Arroyo-Pellicer R, Andrés V. Increased early atherogenesis in young versus old hypercholesterolemic rabbits by a mechanism independent of arterial cell proliferation. *FEBS Letters.* 2002;522:99–103.
  80. Poh M, Boyer M, Solan A, Dahl SLM, Pedrotty D, Banik SSR, McKee JA, Klinger RY, Counter CM, Niklason LE. Blood vessels engineered from human cells. *Lancet.* 2005;365:2122–2124.
  81. Marx N, Wöhrle J, Nussler T, Walcher D, Rinker A, Hombach V, Koenig W, Hoher M. Pioglitazone reduces neointima volume after coronary stent implantation: a randomized, placebo-controlled, double-blind trial in nondiabetic patients. *Circulation.* 2005;112:2792–2798.
  82. Carmeliet P. Angiogenesis in health and disease. *Nat Med.* 2003;9:653–660.
  83. Isner JM. Cancer and atherosclerosis: the broad mandate of angiogenesis. *Circulation.* 1999;99:1653–1655.
  84. Herrmann J, Lerman LO, Mukhopadhyay D, Napoli C, Lerman A. Angiogenesis in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2006;26:1948–1957.
  85. Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, Isner JM. Age-dependent impairment of angiogenesis. *Circulation.* 1999;99:111–120.
  86. Pallini R, Pierconti F, Falchetti ML, D'Arcangelo D, Fernandez E, Maira G, D'Ambrosio E, Larocca LM. Evidence for telomerase involvement in the angiogenesis of astrocytic tumors: expression of human telomerase reverse transcriptase messenger RNA by vascular endothelial cells. *J Neurosurg.* 2001;94:961–971.
  87. Falchetti ML, Pierconti F, Casalbore P, Maggiano N, Levi A, Larocca LM, Pallini R. Glioblastoma induces vascular endothelial cells to express telomerase in vitro. *Cancer Res.* 2003;63:3750–3754.
  88. Yang J, Nagavarapu U, Relloma K, Sjaastad MD, Moss WC, Passaniti A, Herron GS. Telomerized human microvasculature is functional in vivo. *Nat Biotechnol.* 2001;19:219–224.
  89. Murasawa S, Llevadot P, Silver M, Isner JM, Losordo DW, Asahara T. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. *Circulation.* 2002;106:1133–1139.
  90. Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat Med.* 2003;9:369–371.
  91. Yamaguchi Y, Nozawa K, Savovsky E, Hayakawa N, Nimura Y, Yoshida S. Change in telomerase activity of rat organs during growth and aging. *Exp Cell Res.* 1998;242:120–127.
  92. Borges A, Liew CC. Telomerase activity during cardiac development. *J Mol Cell Cardiol.* 1997;29:2717–2724.
  93. Burger AM, Bibby MC, Double JA. Telomerase activity in normal and malignant mammalian tissues: feasibility of telomerase as a target for cancer chemotherapy. *Br J Cancer.* 1997;75:516–522.
  94. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, Schneider MD. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci U S A.* 2001;98:10308–10313.
  95. Oh H, Wang SC, Prahara A, Sano M, Moravec CS, Taffet GE, Michael LH, Youker KA, Entman ML, Schneider MD. Telomere attrition and Chk2 activation in human heart failure. *Proc Natl Acad Sci U S A.* 2003;100:5378–5383.
  96. Beltrami CA, Di Loreto C, Finato N, Rocco M, Artico D, Cigola E, Gambert SR, Olivetti G, Kajstura J, Anversa P. Proliferating cell nuclear antigen (pcna), dna synthesis and mitosis in myocytes following cardiac transplantation in man. *J Mol Cell Cardiol.* 1997;29:2789–2802.

97. Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci U S A*. 1998;95:8801–8805.
98. Anversa P, Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. *Circ Res*. 1998;83:1–14.
99. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med*. 2001;344:1750–1757.
100. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med*. 2002;346:5–15.
101. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003;114:763–776.
102. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003;100:12313–12318.
103. Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal-Ginard B, Leri A, Kajstura J, Quaini E, Anversa P. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2003;100:10440–10445.
104. Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc Natl Acad Sci U S A*. 2005;102:8692–8697.
105. Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, Di Meglio F, Nadal-Ginard B, Frustaci A, Leri A, Maseri A, Anversa P. Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res*. 2003;93:604–613.
106. Leri A, Barlucchi L, Limana F, Deptala A, Darzynkiewicz Z, Hintze TH, Kajstura J, Nadal-Ginard B, Anversa P. Telomerase expression and activity are coupled with myocyte proliferation and preservation of telomeric length in the failing heart. *Proc Natl Acad Sci U S A*. 2001;98:8626–8631.
107. Artandi SE, Attardi LD. Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. *Biochem Biophys Res Commun*. 2005;331:881–890.
108. Samper E, Flores JM, Blasco MA. Restoration of telomerase activity rescues chromosomal instability and premature aging in Terc<sup>-/-</sup> mice with short telomeres. *EMBO Rep*. 2001;2:800–807.
109. Oh H, Schneider MD. The emerging role of telomerase in cardiac muscle cell growth and survival. *J Mol Cell Cardiol*. 2002;34:717–724.
110. Heist EK, Huq F, Hajjar R. Telomerase and the aging heart. *Sci Aging Knowledge Environ*. 2003;2003:PE11.
111. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. *FASEB J*. 1996;10:615–624.
112. Nathan L, Chaudhuri G. Estrogens and atherosclerosis. *Annu Rev Pharmacol Toxicol*. 1997;37:477–515.
113. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med*. 1999;340:1801–1811.
114. Dubey RK, Jackson EK. Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. *Am J Physiol Renal Physiol*. 2001;280:F365–F388.
115. Mooradian AD. Antioxidant properties of steroids. *J Steroid Biochem Mol Biol*. 1993;45:509–511.
116. Tanaka M, Kyo S, Takakura M, Kanaya T, Sagawa T, Yamashita K, Okada Y, Hiyama E, Inoue M. Expression telomerase activity in human endometrium is localized to epithelial glandular cells and regulated in a menstrual phase-dependent manner correlated with cell proliferation. *Am J Pathol*. 1998;153:1985–1991.
117. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A, Inoue M. Estrogen activates telomerase. *Cancer Res*. 1999;59:5917–5921.
118. Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK. Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature*. 2000;407:538–541.
119. Kang SS, Kwon T, Kwon DY, Do SI. Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit. *J Biol Chem*. 1999;274:13085–13090.
120. Doshida M, Ohmichi M, Tsutsumi S, Kawagoe J, Takahashi T, Du B, Mori-Abe A, Ohta T, Saitoh-Sekiguchi M, Takahashi K, Kurachi H. Raloxifene increases proliferation and up-regulates telomerase activity in human umbilical vein endothelial cells. *J Biol Chem*. 2006;281:24270–24278.
121. Imanishi T, Hano T, Nishio I. Estrogen reduces endothelial progenitor cell senescence through augmentation of telomerase activity. *J Hypertens*. 2005;23:1699–1706.
122. Vasa M, Breitschopf K, Zeiher AM, Dimmeler S. Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ Res*. 2000;87:540–542.
123. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000;408:239–247.
124. Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury. Part I: Basic mechanisms and in vivo monitoring of ROS. *Circulation*. 2003;108:1912–1916.
125. Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury. Part II: Animal and human studies. *Circulation*. 2003;108:2034–2040.
126. Breitschopf K, Zeiher AM, Dimmeler S. Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. *FEBS Lett*. 2001;493:21–25.
127. Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci*. 2004;117:2417–2426.
128. Borras C, Esteve JM, Vina JR, Sastre J, Vina J, Pallardo FV. Glutathione regulates telomerase activity in 3T3 fibroblasts. *J Biol Chem*. 2004;279:34332–34335.
129. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest*. 1986;77:1370–1376.
130. Xu D, Neville R, Finkel T. Homocysteine accelerates endothelial cell senescence. *FEBS Lett*. 2000;470:20–24.
131. Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. *Ann NY Acad Sci*. 2004;1019:278–284.
132. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, Kimura M, Larson MG, Meigs JB, Keaney JF, Aviv A. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell*. 2006;5:325–330.
133. Furumoto K, Inoue E, Nagao N, Hiyama E, Miwa N. Age-dependent telomere shortening is slowed down by enrichment of intracellular vitamin C via suppression of oxidative stress. *Life Sci*. 1998;63:935–948.
134. Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, Dimmeler S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ Res*. 2004;94:768–775.
135. Haendeler J, Hoffmann J, Brandes RP, Zeiher AM, Dimmeler S. Hydrogen peroxide triggers nuclear export of telomerase reverse transcriptase via Src kinase family-dependent phosphorylation of tyrosine 707. *Mol Cell Biol*. 2003;23:4598–4610.
136. Wu R, Lamontagne D, de Champlain J. Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. *Circulation*. 2002;105:387–392.
137. Grosser N, Schroder H. Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. *Arterioscler Thromb Vasc Biol*. 2003;23:1345–1351.
138. Bode-Boger SM, Martens-Lobenhoffer J, Tager M, Schroder H, Scalera F. Aspirin reduces endothelial cell senescence. *Biochem Biophys Res Commun*. 2005;334:1226–1232.
139. Imanishi T, Moriaki K, Hano T, Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J Hypertens*. 2005;23:1831–1837.
140. Jeanclos E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, Aviv A. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes*. 1998;47:482–486.
141. Adaikalakoteswari A, Balasubramanyam M, Mohan V. Telomere shortening occurs in Asian Indian type 2 diabetic patients. *Diabetic Med*. 2005;22:1151–1156.
142. Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care*. 2006;29:283–289.

143. Sampson MJ, Hughes DA. Chromosomal telomere attrition as a mechanism for the increased risk of epithelial cancers and senescent phenotypes in type 2 diabetes. *Diabetologia*. 2006;49:1726–1731.
144. Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation*. 1999;99:2192–2217.
145. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A*. 2004;101:17312–17315.
146. Burke A, Fitzgerald GA. Oxidative stress and smoking-induced vascular injury. *Prog Cardiovasc Dis*. 2003;46:79–90.
147. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD. Obesity, cigarette smoking, and telomere length in women. *Lancet*. 2005;366:662–664.
148. Klinger RY, Blum JL, Hearn B, Lebow B, Niklason LE. Relevance and safety of telomerase for human tissue engineering. *Proc Natl Acad Sci U S A*. 2006;103:2500–2505.
149. Hastings R, Qureshi M, Verma R, Lacy PS, Williams B. Telomere attrition and accumulation of senescent cells in cultured human endothelial cells. *Cell Prolif*. 2004;37:317–324.
150. Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, Bronstein A, Chiu CP, Herron GS. Human endothelial cell life extension by telomerase expression. *J Biol Chem*. 1999;274:26141–26148.
151. Trivier E, Kurz DJ, Hong Y, Huang HL, Erusalimsky JD. Differential regulation of telomerase in endothelial cells by fibroblast growth factor-2 and vascular endothelial growth factor-A: association with replicative life span. *Ann NY Acad Sci*. 2004;1019:111–115.
152. Kurz DJ, Hong Y, Trivier E, Huang HL, Decary S, Zang GH, Luscher TF, Erusalimsky JD. Fibroblast growth factor-2, but not vascular endothelial growth factor, upregulates telomerase activity in human endothelial cells. *Arterioscler Thromb Vasc Biol*. 2003;23:748–754.
153. Spyridopoulos I, Haendeler J, Urbich C, Brummendorf TH, Oh H, Schneider MD, Zeiher AM, Dimmeler S. Statins enhance migratory capacity by upregulation of the telomere repeat-binding factor TRF2 in endothelial progenitor cells. *Circulation*. 2004;110:3136–3142.